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In Vitro Bacterial Leakage at the Implant-Abutment Connection of Two Dental Implant Systems with Internal Connection

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A B S T R A C T

Objectives: Bacterial leakage at the implant-abutment interface is one of the main causes of peri-implant inflammation. One of the factors that influences bacterial leakage is the structural design of the interface. Considering the limited studies that have examined slip-joint connections, a comparative study of bacterial leakage was performed on two different systems namely Zimmer (Tapered Screw-Vent, Zimmer Dental) with slip-joint connection and Argon (Konus K3pro, Argon Implants) with conical connection.

Materials and Methods: Twenty-two implants were selected in 2 groups (11 Zimmer with slip-joint connection, and 11 Argon with conical connection) with similar platforms. Escherichia coli (E. coli) suspension (2 µL) was pipetted into the internal lumen of implants. The abutments were screwed onto the implants with a closing torque of 30 Ncm. The assemblies were placed in culture broth for 6, 24, 48 and 72 h, and 7 and 14 days. The colonies were counted and analyzed by the Mann-Whitney test (a=0.05).

Results: Microleakage was observed in 20% of the samples of conical connection group after 6 h to 2 days, and in 50% of the samples in slip-joint connection group after 3 to 7 days. There was a significant difference in bacterial leakage rate between the two implant groups (P<0.001) but no significant difference was seen in bacterial leakage over time (P>0.05).

Conclusion: Type of connection had a significant effect on bacterial leakage, but the rate of bacterial leakage did not significantly change over time.

Keywords: Dental Implants; Dental Leakage; Bacterial Load; Dental Implant-Abutment Design

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INTRODUCTION

In recent years, implant-supported restorations have been widely recognized as the first choice for rehabilitation of edentulous areas due to their optimal esthetics and high success rate [1]. Success in implant therapy depends on the balance between the biological and mechanical factors [2]. Mechanical factors, such as the implant-abutment precise fit, are involved in the success of dental implant rehabilitation [3-5]. Implant-abutment connection misfit also affects the biological factors. The success of dental implants firstly depends on the osseo-integration phenomenon and secondary, preservation of the supporting bone [6-8].

Microscopic gaps created by the implant-abutment misfit enhance the leakage of saliva and oral bacteria, and lead to their accumulation at the implant-abutment connection, resulting in marginal bone loss [9-12]. Marginal bone loss can adversely affect the success of implant treatment. Bacterial leakage through the implant-abutment connection is an important cause of marginal bone loss. Also, the abutment micro-movement under functional forces causes micro-gaps at the implant-abutment connection. Microorganisms colonize the area and penetrate into the inner part of the implant during function, resulting in inflammatory response and eventual implant failure [7, 13, 14].

The structural design of the implant-abutment connection is one factor that affects the microbial leakage. Different connection designs can affect the precise fit and bacterial leakage through the interface [15]. Thus, alternative implant systems with different connection types have been introduced to the market with the main claim of prevention of peri-implant tissue inflammation. According to some studies, internal connections result in precise fit of the implant-abutment connection and are more stable than external connections [16-18]. Internal connection implants have different profiles, including butt Joint, conical, and slip-joint connections [19].

According to the manufacturer, slip-joint connection has an internal bevel (1.5° taper) that starts from the outer part of the platform and extends to the internal part of implant, leading to abutment stability, reduced microleakage, and better horizontal stress distribution, compared with the butt-joint connections [20].

In a systematic review by Schmitt et al, [21] about conical connections, it was shown that conical abutments were better than butt-joint abutments in terms of bacterial seal, microgap, torque retention, and abutment stability. Also, they showed that the success rate and survival rate of conical implants were significantly different from the other types; also, marginal bone loss around implants with conical abutments was lower in the majority of cases.

Considering the fact that microbial leakage is one of the important factors in assessing the precision of fit and quality of implant-abutment connections, and due to the lack of information on slip-joint connections, the present study aimed to compare the bacterial leakage at the implant-abutment connection of two different implant systems namely Zimmer (Tapered Screw-Vent, Zimmer Dental, Carlsbad, CA, USA) and Argon (Konus K3pro, Argon Medical Productions, Germany) with slip-joint and conical connections.

MATERIALS AND METHODS

In this in vitro study, two groups of implants (n=11) were compared based on their connection type. Group 1 included implants with slip-joint connection (Tapered Screw-Vent, 3.7×10, Zimmer Dental, USA) connected to straight titanium abutments.

Fig. 1. Conical connection in Argon implant

Fig. 2. Slip joint connection in Zimmer implant

Group 2 included implants with conical connection (Konus K3pro, 4.5×9, Argon Implants, Kiev,

Ukraine) connected to straight titanium abutments (Figs. 1 and 2). The size of platforms in the two systems was approximately the same. One implant from each group was evaluated as the negative control. The study was approved by the research ethics committee of our university (IR.QUMS.REC.1397.076).

We used all materials in their original sterile packaging. All tests were completed under sterile conditions. The microbiological tests were conducted under the supervision of two blinded experts. In this study, bacterial microleakage was evaluated using the outward method [9,12] (leakage from the internal lumen of the implant to the external environment), and Escherichia coli (E. coli) was used for this purpose [3,6,7,12-17].

First, standard strain E. coli (ATCC 25922) suspension was prepared in trypticase soy broth in 0.5 McFarland standard concentration. Then, the bacterial suspension was diluted to contain 1.5×10^8 colony forming units (CFUs)/mL [12].

Each implant was partially embedded in auto-polymerizing resin (Luxatemp; DGM, Hamburg, Germany) using a custom-made test chamber. This step was performed just to connect the abutments to implants with a torque controller. All mounted implants were autoclave-sterilized for 15 min at 121°C. After several trials, 0.2 µL of 0.5 McFarland (3×10^7 CFUs/mL) suspension was found to be the ideal turbidity of bacterial suspension for inoculation of implant systems.

As a positive control, a test tube was used with only nutrient solution and inoculated with 0.2 µL of E. coli. The turbidity of the suspension, which shows the viability of bacteria, was observed during the procedure. As a negative control, one implant was used in sterile nutrient solution alone. The transparency of the solution confirmed no bacterial contamination.

Before the connection of implants and abutments, the internal lumen of implants was filled with bacterial suspension. In the next step, the abutments were connected to the implants, and an implant torque controller, pre-calibrated at 30 Ncm as recommended by the manufacturer, was used to ensure proper seating torque for all abutments [22].

To prevent microleakage from the abutment screw hole, this area was completely sealed by gutta-percha (Pumadent, Pumadent Co. Ltd., China) and cyanoacrylate adhesive (Razi chemical Co., Tehran, Iran).

In the next step, after removing the implant-abutment assembly from auto-polymerizing acrylic resin, it was ensured that the external surface of the implant was not contaminated. At first, the implant-abutment assemblies were disinfected with 70% alcohol and dried with absorbent papers, and were then placed under a UV lamp for 30 min [23]. Three implants from each group were randomly selected, and samples were collected from the outer surface of the implants with a micro-brush and cultured on MacConkey agar medium. The samples were incubated for 24 h (Memmert, Schwabach, Germany) at 37°C [24].

Finally, sterile trypticase soy broth liquid medium was poured to completely cover the implant-abutment interface. The medium level was lower than the screw hole on the top of the abutment because the screw hole could act as a second bacterial reservoir (Fig. 3).

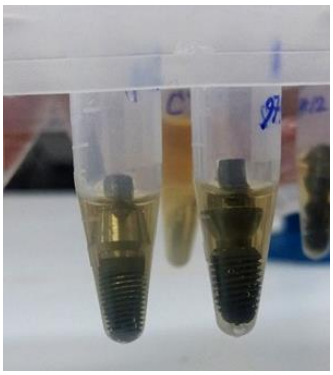

Fig. 3. Implant-abutment assembly in tryptic soy broth medium

The specimens were placed in an incubator and kept at 37°C for 14 days. Bacterial leakage was assessed by observing the medium turbidity at 6, 24, 48 and 72 h, and 7 and 14 days. Samples were collected from the trypticase soy broth liquid medium and cultured on MacConkey agar [25](Fig. 4). The plates were incubated at 37°C under suitable conditions and after 24 h, the colony count was measured and reported as CFUs/mL. The Kolmogorov-Smirnov test was used to analyze data distribution.

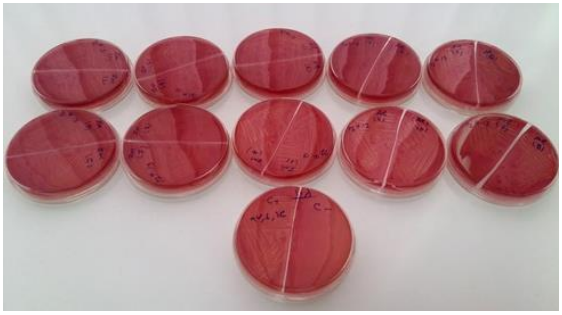

Fig. 4. Cultivation in MacConkey agar medium

The Mann-Whitney test was used for the comparisons using SPSS 21 (SPSS Inc., IL, USA) at 0.05 level of significance.

RESULTS

Since the number of microbial colonies plays an important role in development and severity of peri-implantitis, in this study, instead of comparing the presence/absence of contamination, the number of colonies in each group was counted and compared with the Man-Whitney test [26]. The results of microbial leakage assessment at the implant-abutment connection showed that in the Zimmer system with slip-joint connection, 2 of 10 samples (20%) and in the Argon system with a conical connection, 5 of 10 samples (50%) had microbial leakage.

Table 1. Colony count (CFUs/mL) in conical connection specimens at different time points

Sample	Time					
	6 h	24 h	48 h	72 h	1 w	2 w
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	100	10^5	10^5	10^5	10^5	10^5
4	0	200	10^5	10^5	10^5	10^5
5	0	0	300	10^5	10^5	10^5
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	400	10^5	10^5	10^5	10^5	10^5
9	0	0	0	0	0	0
10	0	0	200	10^5	10^5	10^5

h: hours; w: weeks

The results of the colony count (CFUs/mL) at different time points in case of microbial leakage showed medium turbidity after 3 to 7 days in slip-joint connection, and after 6 h to 2 days in conical connection (Tables 1 and 2).

Table 2. Colony count (CFUs/mL) in slip-joint connection specimens at different time points

Sample	Time					
	6 h	24 h	48 h	72 h	1 w	2 w
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	400	10^5	10^5
8	0	0	0	0	0	0
9	0	0	0	0	300	10^5
10	0	0	0	0	0	0

h: hours; w: weeks

Comparing the number of colony count in slip-joint and conical connections:

The results of the Mann-Whitney test showed that contamination in the implant group with conical connection at 48 h (P=0.013) and 72 h (P=0.034) was significantly more than that in the slip-joint connection but this difference was not significant at other time points (P=0.147, 0.068, 0.114 and 0.170 at 6 h, 24 h, 1 week and 2 weeks, respectively).

However, there was a significant difference in bacterial leakage rates between the two implant groups (P<0.001). In other words, implant connection had a significant effect on the mean rank of bacterial leakage, which was 70.66 in implants with conical connection and 50.34 in implants with slip-joint connection.

Comparing the number of colonies over time in the slip-joint and conical connection types:

Since the data did not have a normal distribution (Kolmogorov-Smirnov test), the Kruskal-Wallis non-parametric test, was used for comparison. The mean rank of the bacterial leakage for implants with conical and slip-joint connections was minimum at 6 h and maximum at 2 weeks.

DISCUSSION

In the present study, microbial leakage was compared in two different implant systems with two different profiles at implant-abutment connections. A former study showed that success and survival rates of implant treatment in systems with conical connections

were comparable to other systems while bone resorption was less than that in other connection types [21].

Conical connection has higher mechanical stability and longer clinical success than butt-Joint connection [20]. On the other hand, the precision of implant-abutment connection with slip-joint profile and its microbial leakage have been questioned compared with other connection types. Therefore, the Zimmer implant system was used to evaluate the slip-joint connection compared with the Argon implant with the conical connection in this study.

The methods used to evaluate microbial leakage are categorized into two groups: The inward method, which is leakage from the external part of the implant towards the internal part [17,27,28], and the outward method, which is leakage from the internal part towards the external part [9,12,27-31]. Both methods have their own specific sensitivities that can lead to false positive or false negative results. The inward method better simulates the in vivo conditions, but this method has some disadvantages. In this method, the microleakage of the implant-abutment assembly should be confirmed by the presence of bacteria in the internal lumen of the implant. For this reason, the implant-abutment assembly should be opened after a specific period of time; thus, the test can only be performed once because of the increased probability of contamination of components during opening and closing of abutments. Repetition of the test increases the error rate, which is followed by a false positive result. Therefore, evaluation of the long-term growth rate of bacteria in the above-mentioned method is not possible, in contrast to the outward method [29]. Disinfection of the implant surface and the abutment can also result in false negative results because of the possibility of material leakage into it [32].

Based on the hypothesis that microbial microleakage from the inside towards the outside could also be reversed, this study was conducted to test the microleakage by the outward method. In this study, E. coli was used to evaluate bacterial leakage, which is Gram-negative and has a diameter of 1.1 to 1.5 µm. It can also leak through the implant-abutment connection. E. coli is a facultative anaerobe and has the ability to survive in inappropriate conditions as in implant's internal cavity with limited food and oxygen for 14 days. Also, E. coli is used in in vitro studies because it is commonly isolated from peri-implantitis lesions [32], and has been used in many studies on implant leakage [29].

The present study was designed to investigate microbial microleakage at 14 days, which is sufficient for bacteria to leak through the implant-abutment connection. Aloise et al. [32] showed that bacterial colonization occurs 14 days after implant placement.

In the present study, the results showed that in the Zimmer system with slip-joint connection, 2 samples of 10 (20%) and in the Argon system with a conical connection, 5 samples of 10 (50%) had microbial leakage. The results of counting colonies at different time points showed that contamination in the implant group with a slip-joint connection occurred after 3 to 7 days while it occurred 6 h to 2 days later in the implant group with conical connection. In other words, contamination occurred in more cases and in a shorter period of time in the conical implant group.

Various studies [9,10,12,15,20,22] have investigated microbial microleakage at the implant-abutment connection. Microbial microleakage rates are highly variable depending on the methodology of studies.

The study by Nassar and Abdalla [33] was the only study that compared slip-joint connection in two geometries of internal hexagon and trilobe connection, and indicated that greater leakage occurred in hexagon connection than trilobe. It appears that the gap dimension at the implant-abutment connection of different systems, which ranges from 20 µm to 49 µm, is the reason for different results. Harder et al. [30], compared conical connections and showed that in the Ankylos group, all samples were infected after 5 min while in the Astratech group, 87.5% of the samples were infected before 7 days. Their results, similar to ours, showed that microbial microleakage in conical connection can be detected in a short time. In

a study by Tesmer et al [22] the rate of microbial leakage in the conical connection was much lower than that in butt-joint connection. After 5 days, only 3 samples of 10 in the conical connection group were infected; while, in the butt-joint group, 9 of 10 samples were infected. Contamination in the conical implant group in our study was more frequent than that in the similar implant group in the study by Tesmer et al, [22] which could be due to the differences in the precision of components in the systems, the type of bacteria, and different methods of bacterial microleakage assessment (outward method).

Sperandio and Napimoga [34] used the outward technique to investigate the microbial leakage of conical connections. The results showed that half of the samples were contaminated after 14 days. Their results were similar to those of the present study.

In general, studies have shown that conical connections have lower leakage rate than butt-joint connections [23, 35]. Variations in the results can be due to the type of implant system and the precision of components as well as the method of microleakage assessment. But no previous study has compared this connection type with slip-joint connection.

There was no significant difference in the amount of bacterial leakage over time in any of the two implant groups in our study, (P=0.338). In other words, time had a significant effect on bacterial leakage. The results of this study showed that bacterial leakage rate was significantly different between the two implant groups. In other words, the type of connection had a significant effect on bacterial leakage rate but the rate of bacterial leakage did not significantly change over time. The apparently better results of the slip-joint connection compared with conical connection type may be due to the internal bevel in the former connection, which is machined to provide a slip-fit in the conical portion of the coupling and a 1° taper in the hex portion to provide a friction-fit.

This connection reduces horizontal stresses better than the flat "butt-joint" connection that leads to greater abutment stability and decreased leakage [36], which, of course needs to be investigated in further studies under dynamic loading. Also, due to the similarity of the design of this connection with butt-joint, it would be useful to compare these two connections.

CONCLUSION

Type of connection had a significant effect on bacterial leakage, but the rate of bacterial leakage did not significantly change over time.

CONFLICT OF INTEREST STATEMENT

None declared.

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